

WHAT IS CLAIMED IS:

1. A method of producing lactic acid, comprising:
 - aerobically culturing in a first culture medium an acid-tolerant (AT) yeast strain that produces essentially no ethanol when cultured in a culture medium,
 - 5 wherein the AT yeast strain comprises a genome that comprises an exogenous lactate dehydrogenase gene that is capable of being expressed in the AT yeast strain, wherein a protein resulting from the expression has lactate dehydrogenase activity,
 - 10 wherein the AT yeast strain is capable of growing in a minimal medium at a lower pH than a parent yeast strain.
2. The method of claim 1, wherein the AT yeast strain is C₂ carbon source independent and is capable of producing lactic acid at a pH of less than about 3.5.
- 15 3. The method of claim 1, wherein the AT yeast strain is C₂ carbon source independent and is capable of producing lactic acid at a pH of less than about 2.8.
4. The method of claim 1, wherein the AT yeast strain is C₂ carbon source independent and is capable of producing lactic acid at a pH of less than about 2.3.
- 20 5. The method of claim 1, wherein the AT yeast strain is capable of producing greater than about 50 grams lactic acid/100 grams glucose when cultured in the minimal medium comprising glucose as a sole carbon source.
- 25 6. The method of claim 1, wherein the AT yeast strain is capable of producing between about 50 and 85 grams lactic acid/100 grams glucose when cultured in the minimal medium comprising glucose as a sole carbon source.
7. The method of claim 1, wherein the AT yeast strain is capable of producing 30 between about 70 and 85 grams lactic acid/100 grams glucose when cultured in the minimal medium comprising glucose as a sole carbon source.

8. The method of claim 1, wherein a culture broth resulting from the culturing of the AT yeast strain comprises less ppm of at least one of glycerol, erythritol, malic acid, pyruvic acid, succinic acid, formic acid, and fumaric acid than a culture broth resulting

5 from the culturing of the parent strain in essentially the same minimal medium under essentially the same culture conditions.

9. The method of claim 1, wherein the AT yeast strain belongs to a genus selected from the group consisting of *Saccharomyces*, *Candida*, *Schizosaccharomyces*, and

10 *Kluyveromyces*.

10. The method of claim 1, wherein the AT yeast strain is a *Saccharomyces cerevisiae*.

15 11. The method of claim 1, wherein the AT yeast strain is a *Saccharomyces cerevisiae* that has a genotype pdc1(-6, -2)::loxP pdc5(-6,-2)::loxP pdc6(-6,-2)::loxP ura3-52 YEpLpLDH.

12. The method of claim 1, wherein the culturing is performed in an aerobic batch
20 culture, in an aerobic fed-batch culture, or in an aerobic chemostat.

13. The method of claim 1, wherein the AT yeast strain is C₂ carbon source-independent.

25 14. The method of claim 13, wherein the first culture medium is a minimal medium comprising at least one defined carbon source selected from the group consisting of glucose, sucrose, fructose, maltose, lactose, and galactose.

15. The method of claim 14, wherein glucose is the sole carbon source.

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16. The method of claim 1, wherein the AT yeast strain is C₂ carbon source-dependent and the first culture medium is a minimal medium comprising a carbon source consisting essentially of glucose and at least one C₂ carbon source.

5 17. The method of claim 1, wherein the first culture medium consists essentially of at least one defined carbon source, at least one nitrogen source, monopotassium phosphate, magnesium sulfate, copper sulfate, ferric chloride, manganese sulfate, sodium molybdate, zinc sulphate, biotin, inositol, thiamine, and water, wherein the nitrogen source is selected from the group consisting of urea, ammonium sulfate, ammonium phosphate, 10 and ammonium nitrate.

18. The method of claim 1, wherein a chromosome of the AT yeast strain comprises the exogenous lactate dehydrogenase gene.

15 19. The method of claim 1, wherein at least one plasmid comprising the exogenous lactate dehydrogenase gene is present in the AT yeast strain.

20. The method of claim 1, wherein the exogenous lactate dehydrogenase gene is a *Lactobacillus plantarum*, bovine, *Lactobacillus casei*, *Bacillus megaterium*, *Rhizopus oryzae*, or *Bacillus stearothermophilus* lactate dehydrogenase gene.

21. The method of claim 1, wherein the exogenous lactate dehydrogenase gene is a *Lactobacillus plantarum* lactate dehydrogenase gene.

25 22. The method of claim 1, further comprising the step of recovering and purifying the lactic acid or a salt thereof.

23. The method of claim 22, wherein the purification step comprises at least one of distillation, ion exchange, nanofiltration or solvent extraction.

24. An acid-tolerant (AT) yeast strain that produces essentially no ethanol when cultured in a culture medium,

wherein the AT yeast strain comprises a genome that comprises an exogenous lactate dehydrogenase gene that is capable of being expressed in the AT yeast strain,

5 wherein a protein resulting from the expression has lactate dehydrogenase activity,

wherein the AT yeast strain is capable of producing lactic acid in a minimal medium at a lower pH than a parent yeast strain.

10 25. The AT yeast strain of claim 24, wherein the AT yeast strain has no detectable amount of pyruvate decarboxylase activity.

26. The AT yeast strain of claim 25, wherein a wild type strain of the AT yeast strain is Crabtree positive.

15 27. The AT yeast strain of claim 24, wherein the AT yeast strain is capable of producing lactic acid at a pH of less than about 3.5.

20 28. The AT yeast strain of claim 24, wherein the AT yeast strain is capable of producing lactic acid at a pH of less than about 2.8.

29. The AT yeast strain of claim 24, wherein the AT yeast strain is capable of producing lactic acid at a pH of less than about 2.3.

25 30. The AT yeast strain of claim 24, wherein the AT yeast strain is capable of producing greater than about 500 mM lactic acid in a culture broth, when cultured aerobically in a minimal medium.

30 31. The AT yeast strain of claim 30, wherein the AT yeast strain is capable of producing greater than about 565 mM lactic acid.

32. The AT yeast strain of claim 30, wherein the AT yeast strain is capable of producing greater than about 665 mM lactic acid.

5 33. The AT yeast strain of claim 24, wherein the AT yeast strain belongs to a genus selected from the group consisting of *Saccharomyces*, *Candida*, *Schizosaccharomyces*, *Torulaspora*, *Kluyveromyces*, *Zygosaccharomyces* and *Dekkera*.

10 34. The AT yeast strain of claim 24, wherein the AT yeast strain belongs to a genus selected from the group consisting of *Saccharomyces*, *Candida*, *Schizosaccharomyces*, and *Kluyveromyces*.

35. The AT yeast strain of claim 24, wherein the AT yeast strain belongs to the genus *Saccharomyces*.

15 36. The AT yeast strain of claim 24, wherein the AT yeast strain is a *Saccharomyces cerevisiae*.

20 37. The AT yeast strain of claim 24, wherein the AT yeast strain is a *Saccharomyces cerevisiae* that has a genotype pdc1(-6, -2)::loxP pdc5(-6,-2)::loxP pdc6(-6,-2)::loxP ura3-52 YEpLpLDH.

38. The AT yeast strain of claim 24, wherein the AT yeast strain is a *Kluyveromyces thermotolerans*, a *Zygosaccharomyces bailii*, a *Schizosaccharomyces pombe*, or a *Candida glabrata*.

25 39. The AT yeast strain of claim 24, wherein the AT yeast strain is C₂ carbon source-independent.

30 40. The AT yeast strain of claim 39, wherein the AT yeast strain is capable of growing in a minimal medium comprising at least one defined carbon source selected from the group consisting of glucose, sucrose, fructose, maltose, lactose, and galactose.

41. The AT yeast strain of claim 39, wherein the AT yeast strain is capable of growing in a minimal medium comprising glucose as the sole carbon source.

5 42. The AT yeast strain of claim 24, wherein the AT yeast strain is C₂ carbon source-dependent and the AT yeast strain is capable of growing in a minimal medium comprising a carbon source consisting essentially of glucose and at least one C₂ carbon source.

10 43. The AT yeast strain of claim 24, wherein the AT yeast strain is capable of growing in a mineral medium consisting essentially of at least one defined carbon source, at least one defined nitrogen source, monopotassium phosphate, magnesium sulfate, copper sulfate, ferric chloride, manganese sulfate, sodium molybdate, zinc sulphate, biotin, inositol, thiamine, and water, wherein the defined nitrogen source is selected from 15 the group consisting of urea, ammonium sulfate, ammonium phosphate, and ammonium nitrate.

44. The AT yeast strain of claim 24, wherein a chromosome of the AT yeast strain comprises the exogenous lactate dehydrogenase gene.

20 45. The AT yeast strain of claim 24, wherein at least one plasmid comprising the exogenous lactate dehydrogenase gene is present in the AT yeast strain.

25 46. The AT yeast strain of claim 24, wherein the exogenous lactate dehydrogenase gene is functionally linked to a promoter.

47. The AT yeast strain of claim 46, wherein the promoter is a triose phosphate isomerase promoter.

48. The AT yeast strain of claim 46, wherein the promoter is selected from the group consisting of pyruvate decarboxylase promoters, alcohol dehydrogenase promoters, and L-threonine dehydrogenase promoters.

5 49. The AT yeast strain of claim 46, wherein the promoter is a *Kluyveromyces* pyruvate decarboxylase promoter.

10 50. The AT yeast strain of claim 24, wherein the exogenous lactate dehydrogenase gene is a *Lactobacillus plantarum*, bovine, *Lactobacillus casei*, *Bacillus megaterium*, *Rhizopus oryzae*, or *Bacillus stearothermophilus* lactate dehydrogenase gene.

51. The AT yeast strain of claim 24, wherein the exogenous lactate dehydrogenase gene is a *Lactobacillus plantarum* lactate dehydrogenase gene.

15 52. The AT yeast strain of claim 24, wherein the AT yeast strain is capable of producing lactic acid consisting essentially of L-lactic acid.

53. An acid-tolerant (AT) *S. cerevisiae* that produces essentially no ethanol when cultured in a culture medium,

20 wherein the AT *S. cerevisiae* comprises a genome that comprises an exogenous lactate dehydrogenase gene that is capable of being expressed in the AT *S. cerevisiae*, wherein a protein resulting from the expression has lactate dehydrogenase activity,

25 wherein the AT *S. cerevisiae* is capable of producing lactic acid in a minimal medium at a lower pH than a parent *S. cerevisiae* strain.

54. The acid-tolerant (AT) *S. cerevisiae* of claim 53, wherein the AT *S. cerevisiae* has no detectable amount of pyruvate decarboxylase activity.

30 55. The acid-tolerant (AT) *S. cerevisiae* of claim 53, wherein the exogenous lactate dehydrogenase gene is a *Lactobacillus plantarum* lactate dehydrogenase gene.

56. The acid-tolerant (AT) *S. cerevisiae* of claim 53, wherein at least one plasmid in the AT *S. cerevisiae* comprises the exogenous lactate dehydrogenase gene.

5 57. The AT *S. cerevisiae* of claim 53, wherein the AT *S. cerevisiae* has a genotype pdc1(-6, -2)::loxP pdc5(-6,-2)::loxP pdc6(-6,-2)::loxP ura3-52 YEplpLDH.

10 58. The AT *S. cerevisiae* of claim 53, wherein the AT *S. cerevisiae* is capable of producing greater than about 500 mM lactic acid in a culture broth, when cultured aerobically in a minimal medium.

59. An acid-tolerant C₂ carbon source-independent (CI) yeast strain that produces essentially no ethanol when cultured in a culture medium,

15 wherein the genome of the yeast strain comprises an exogenous lactate dehydrogenase gene that is capable of being expressed,

wherein a protein resulting from the expression has lactate dehydrogenase activity,

wherein the yeast is capable of producing lactic acid when cultured under aerobic conditions in a first minimal medium comprising glucose as a sole carbon source; and

20 wherein the CI yeast strain is capable of producing lactic acid in the first minimal medium at a lower pH than a parent strain.

60. The CI yeast strain of claim 59, wherein the CI yeast strain has no detectable amount of pyruvate decarboxylase activity.

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61. The CI yeast of strain of claim 60, wherein a wild type yeast of the same strain is Crabtree positive.

30 62. The CI yeast strain of claim 59, wherein the exogenous lactate dehydrogenase gene is a *Lactobacillus plantarum*, bovine, *Lactobacillus casei*, *Bacillus megaterium*, *Rhizopus oryzae*, or *Bacillus stearothermophilus* lactate dehydrogenase gene.

63. The CI yeast strain of claim 59, wherein the exogenous lactate dehydrogenase gene is a *Lactobacillus plantarum* lactate dehydrogenase gene.

5 64. The CI yeast strain of claim 59, wherein a chromosome of the CI yeast strain comprises the exogenous lactate dehydrogenase gene.

65. The CI yeast strain of claim 59, wherein at least one plasmid comprising the exogenous lactate dehydrogenase gene is present in the CI yeast strain.

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66. The CI yeast strain of claim 65, wherein the at least one plasmid is a 2 micron plasmid.

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67. The CI yeast strain of claim 59, wherein the CI yeast strain is capable of producing lactic acid at a pH of less than about 2.8.

68. The CI yeast strain of claim 59, wherein the CI yeast strain is capable of producing lactic acid at a pH of less than about 2.3.

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69. The CI yeast strain of claim 59, wherein the CI yeast strain is capable of producing greater than about 50 grams lactic acid/100 grams glucose when cultured in the minimal medium.

70. The CI yeast strain of claim 59, wherein the CI yeast strain is capable of producing between about 50 and 85 grams lactic acid/100 grams glucose when cultured in the minimal medium.

71. The CI yeast strain of claim 59, wherein the CI yeast strain is capable of producing between about 70 and 85 grams lactic acid/100 grams glucose when cultured in the minimal medium.

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72. The CI yeast strain of claim 59, wherein the CI yeast strain is capable of producing greater than about 565 mM lactic acid in a culture broth, when cultured aerobically in a second minimal medium.

5 73. The CI yeast strain of claim 72, wherein the CI yeast strain is capable of producing greater than about 665 mM lactic acid.

10 74. The CI yeast strain of claim 59, wherein the CI yeast strain belongs to a genus selected from the group consisting of *Saccharomyces*, *Candida*, *Schizosaccharomyces*, *Torulaspora*, *Kluyveromyces*, *Zygosaccharomyces* and *Dekkera*.

75. The CI yeast strain of claim 59, wherein the CI yeast strain belongs to a genus selected from the group consisting of *Saccharomyces*, *Candida*, *Schizosaccharomyces*, and *Kluyveromyces*.

15 76. The CI yeast strain of claim 59, wherein the CI yeast strain is selected from the group consisting of *Saccharomyces cerevisiae*, *Kluyveromyces thermotolerans*, *Zygosaccharomyces bailii*, *Schizosaccharomyces pombe*, and *Candida glabrata*

20 77. The CI yeast strain of claim 59, wherein the CI yeast strain is a *Saccharomyces cerevisiae* that has a genotype pdc1(-6, -2)::loxP pdc5(-6,-2)::loxP pdc6(-6,-2)::loxP ura3-52 YEpLpLDH.

25 78. The CI yeast strain of claim 59, wherein the CI yeast strain is capable of growing in a second minimal medium comprising at least one defined carbon source selected from the group consisting of glucose, sucrose, fructose, maltose, lactose, and galactose.

30 79. The CI yeast strain of claim 59, wherein the CI yeast strain is capable of growing in a second minimal medium consisting essentially of at least one defined carbon source selected from the group consisting of glucose, sucrose, fructose, maltose, lactose, and galactose; at least one nitrogen source selected from the group consisting of urea,

ammonium phosphate, ammonium nitrate, and ammonium sulfate; monopotassium phosphate; magnesium sulfate; copper sulfate; ferric chloride; manganese sulfate; sodium molybdate; zinc sulphate; biotin; inositol; thiamine; and water.

- 5 80. A minimal culture medium consisting essentially of,
 at least one defined carbon source; at least one nitrogen source, monopotassium phosphate, magnesium sulfate, copper sulfate, ferric chloride, manganese sulfate, sodium molybdate, zinc sulphate, biotin, inositol, thiamine, and water.

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81. The minimal culture medium of claim 80, wherein the defined carbon source comprises a C₂ carbon source.

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82. The minimal culture medium of claim 81, wherein the defined carbon source further comprises at least one compound selected from the group consisting of glucose, sucrose, fructose, lactose, galactose, and maltose.

83. The minimal culture medium of claim 80, wherein glucose is the sole carbon source.

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84. The minimal culture medium of claim 80, wherein the minimal culture medium comprises between about 5 g glucose/liter and 100g glucose/liter.

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85. The minimal culture medium of claim 84, wherein the minimal culture medium comprises between 0.1 wt% ethanol and 1 wt% ethanol.

86. The minimal culture medium of claim 80, wherein the minimal culture medium comprises calcium carbonate.

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87. The minimal culture medium of claim 80, wherein the nitrogen source comprises at least one compound selected from the group consisting of urea, ammonium sulfate, ammonium nitrate, and ammonium phosphate.

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88. The minimal culture medium of claim 80, wherein the minimal culture medium comprises between about 0.5 and 5 g ammonium sulfate /liter.

89. The minimal culture medium of claim 80, wherein the minimal culture medium
10 comprises between about 0.5 and 2 g ammonium sulfate/liter.

90. The minimal culture medium of claim 80, wherein the minimal culture medium comprises between about 1 and 2 g ammonium sulfate/liter.

15 91. The minimal culture medium of claim 80, wherein the minimal culture medium comprises between about 0.1 and 2 g urea /liter.

92. The minimal culture medium of claim 80, wherein the minimal culture medium comprises between about 0.1 and 1 g urea /liter.

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93. The minimal culture medium of claim 80, wherein the minimal culture medium comprises between about 0.5 and 2 g urea /liter.

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94. The minimal culture medium of claim 80, wherein the minimal culture medium comprises between about 0.2 and 2 g monopotassium phosphate /liter; between about 0.1 and 1g magnesium sulfate/liter; between about 5 and 50 micrograms copper sulfate/liter; between about 0.05 and 0.25 mg ferric chloride/liter; between about 0.05 and 0.5 mg manganese sulfate/liter; between about 0.05 and 0.25 mg sodium molybdate/liter; between about 0.05 and 0.5 mg zinc sulphate/liter; between about 0.5 and 2.5 micrograms biotin/liter; between about 0.5 and 4 mg inositol/liter; and between about 0.05 and 0.5 mg thiamine/liter .

95. The minimal culture medium of claim 80, wherein the minimal culture medium comprises between about 5 g glucose/liter and 100g glucose/liter or between about 0.1 wt% and 1 wt% ethanol, about 5 g ammonium sulfate/liter or about 1 g urea/liter, about 1 g monopotassium phosphate/liter, about 0.5 g magnesium sulfate/liter, about 40 micrograms copper sulfate/liter, about 0.2 mg ferric chloride/liter, about 0.4 mg manganese sulfate/liter, about 0.2 mg sodium molybdate/liter, about 0.4 mg zinc sulphate/liter, about 2 micrograms biotin/liter, about 2 mg inositol/liter, and about 0.4 mg thiamine/liter.

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96. A recombinant yeast strain having a genome comprising an exogenous lactate dehydrogenase gene that is capable of being expressed in the recombinant yeast strain, wherein a protein resulting from the expression has lactate dehydrogenase activity,

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wherein the recombinant yeast strain is capable of producing at least about 50 grams lactic acid/100 grams glucose when grown in a minimal medium comprising glucose as the sole carbon source, and

wherein the recombinant yeast strain is capable of growing at a pH of less than about 3.5.

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97. The recombinant yeast strain of claim 96, wherein the recombinant yeast strain is capable of producing between about 50 and 85 grams lactic acid/100 grams glucose when grown in the minimal medium.

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98. The recombinant yeast strain of claim 96, wherein the recombinant yeast strain is capable of producing between about 70 and 85 grams lactic acid/100 grams glucose when grown in the minimal medium.

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99. The recombinant yeast strain of claim 96, wherein the recombinant yeast strain is capable of producing lactic acid at a pH of less than about 2.8.

100. The recombinant yeast strain of claim 96, wherein the recombinant yeast strain is capable of producing lactic acid at a pH of less than about 2.3.

101. The recombinant yeast strain of claim 96, wherein the recombinant yeast strain is capable of producing lactic acid at a pH of less than about 2.0.

102. A method of producing lactic acid, comprising:

aerobically culturing in a first culture medium a recombinant yeast strain having a genome comprising an exogenous lactate dehydrogenase gene that is capable of being expressed in the recombinant yeast strain, wherein a protein resulting from the expression has lactate dehydrogenase activity, wherein the recombinant yeast strain is capable of producing at least about 50 grams lactic acid/100 grams glucose when grown in a minimal medium comprising glucose as the sole carbon source, and wherein the recombinant yeast strain is capable of growing at a pH of less than about 3.5.

15 103. An acid tolerant (AT) yeast strain that is recovered by a selection process comprising:

(a) growing a first yeast strain in a first aerobic culture, wherein the first aerobic culture is started by inoculating a first minimal medium with a first yeast strain that produces essentially no ethanol when cultured in a culture medium, wherein the first yeast strain comprises a genome that comprises an exogenous lactate dehydrogenase gene that is capable of being expressed in the first yeast strain, wherein a protein resulting from the expression has lactate dehydrogenase activity, wherein during the growth of the first aerobic culture the pH of the culture decreases,

20 25 (b) determining about the lowest pH at which the first yeast strain is still capable of growing in the first minimal medium, and

(c) recovering at least one second yeast strain from the first aerobic culture, when the first aerobic culture is still growing, and the pH is about at its lowest.

30 104. The AT yeast strain of claim 103, wherein the first yeast strain lacks at least one of pyruvate decarboxylase enzyme activity or alcohol dehydrogenase enzyme activity.

105. The AT yeast strain of claim 104, wherein a wild type yeast strain for the first yeast strain is Crabtree positive.

5 106. The AT yeast strain of claim 103, wherein the selection process further comprises the steps of

(d) growing a second aerobic culture, wherein the second aerobic culture is started by inoculating fresh minimal medium with the recovered second yeast strain wherein during the growth of the second aerobic culture the pH of the culture decreases, and

10 (e) recovering at least one third yeast strain from the second aerobic culture, when the second aerobic culture is still growing, and the pH is less than about the lowest pH of the first aerobic culture.

15 107. The AT yeast strain of claim 106, wherein the selection process further comprises repeating steps (d) and (e) at least one time comprising inoculating the fresh minimal medium with a yeast strain recovered from the previous repetition.

20 108. The AT yeast strain of claim 107, wherein the about lowest pH of the aerobic culture at which the AT yeast strain is growing of the last repetition is less than about the lowest pH of the aerobic culture at which the AT yeast strain is growing of the previous repetition.

109. An acid-tolerant C₂ carbon source-independent (CI) yeast strain that is recovered by a selection process comprising:

25 (a) inoculating a minimal medium with a first yeast strain having that produces essentially no ethanol when cultured in a culture medium and that requires a C₂ carbon source when glucose is the only other carbon source in the minimal medium , wherein the genome of the yeast strain comprises an exogenous lactate dehydrogenase gene that is capable of being expressed, wherein a protein resulting from the expression has lactate dehydrogenase activity, wherein the yeast is capable of producing lactic acid or salts thereof when cultured under aerobic conditions in a first minimal medium comprising

glucose and a C₂ carbon source; and wherein the first yeast strain is capable of growing in the first minimal medium at a lower pH than its parent strain,

- (b) culturing the first yeast strain in a series of aerobic batch cultures using a second minimal medium, wherein at the start of the series the second minimal medium 5 comprises glucose and a C₂ carbon source as the sole carbon sources and at concentrations sufficient to permit growth of the yeast culture, wherein the concentration of the C₂ carbon source is decreased over the series of batch cultures, and wherein each successive batch culture is seeded with yeast grown in a batch culture from earlier in the series; and
- 10 (c) recovering at least one CI yeast strain from the series of batch cultures that is capable of growing without a C₂ carbon source and with glucose as a sole carbon source.

110. The CI yeast strain of claim 109, wherein the first yeast strain lacks at least one of pyruvate decarboxylase enzyme activity or alcohol dehydrogenase enzyme activity.

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111. The CI yeast strain of claim 109, wherein a wild type yeast of the first yeast strain is Crabtree positive.

112. An yeast strain having a deposit number NRRL Y-30696.

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113. An yeast strain having a deposit number NRRL Y-30698.

114. A culture medium consisting essentially of water, about 70 g/liter glucose, about 0.5 wt% ethanol, about 1 g/liter urea, about 1 g/liter monopotassium phosphate, about 0.5

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g/liter magnesium sulfate heptahydrate, about 2.78 g/liter calcium carbonate, about 62.5 micrograms/liter copper sulfate pentahydrate, about 200 micrograms/liter ferric chloride, about 450 micrograms/liter manganese sulfate monohydrate, about 235 micrograms/liter sodium molybdate dihydrate, about 712 micrograms/liter zinc sulfate heptahydrate, 2 micrograms/liter biotin, 2000 micrograms/liter inositol, and 400 micrograms/liter thiamine hydrochloride.

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115. A culture medium comprising between about 400 and 1100 ppm N, between
about 215 and 287 ppm K⁺, between about 525 and 700 ppm PO₄⁻², about 49 ppm of
Mg⁺², about 195 ppm SO₄⁻², about 1100 ppm of Ca⁺², about 0.07 ppm Fe⁺³, about 0.145
ppm Mn⁺², about 0.09 ppm Mo⁻⁴, about 0.16 ppm Zn⁺², about 0.015 ppm Cu⁺², about

5 0.002 mg/liter biotin, about 2 mg/liter inositol, and about 0.4 mg/liter thiamine
hydrochloride.

116. A fermentation broth comprising:

at least about 500 mM lactic acid and a first group of compounds,

10 wherein the ratio of the mM lactic acid to mM of the first group of compounds in
the fermentation is at least about 54, wherein the first group of compounds consists of
glycerol, erythritol, mannitol, malic acid, pyruvic acid, succinic acid, formic acid, and
fumaric acid.

15 117. The fermentation broth of claim 116, wherein the pH of the fermentation broth is
between about 2.3 and 2.4.

118. The fermentation broth of claim 116, wherein the broth comprises at least about
565 mM lactic acid.

20 119. The fermentation broth of claim 116, wherein the broth comprises at least about
665 mM lactic acid.

120. The fermentation broth of claim 116, wherein the ratio is greater than about 66.

25 121. The fermentation broth of claim 116, wherein the ratio is greater than about 184.

122. The fermentation broth of claim 116, wherein the fermentation broth is a product
of fermentation by a *S. cerevisiae* strain.

30 123. A yeast plasmid comprising a yeast replication origin and a *Lactobacillus* lactate
dehydrogenase gene functionally linked to a promoter.

124. The plasmid of claim 122, wherein the replication origin is a 2 micron replication origin.

5 125. The plasmid of claim 122, wherein the lactate dehydrogenase gene is a L-lactate dehydrogenase gene.

126. The plasmid of claim 122, wherein the lactate dehydrogenase gene is a *Lactobacillus plantarum* lactate dehydrogenase gene.

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127. The plasmid of claim 122, wherein the promoter is a triose phosphate isomerase promoter

128. The plasmid of claim 122, wherein the plasmid is YEpLpLDH.